植物研究雑誌
J. Jpn. Bot.
81: 113–120 (2006)

Homostyly and Autogamy in *Ophiorrhiza pumila* (Rubiaceae) from the Ryukyu Archipelago

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(Received on September 15, 2005)

Floral morphology of *Ophiorrhiza pumila* from the Ryukyus was investigated to determine the presence/absence of heterostyly. The investigation indicated that *O. pumila* is not distylous, but is long-styled homostylous, with stigmata and anthers at almost the same height around the throat of corolla tubes. This agrees with a previous description of *O. pumila* from continental China, but differs from that of heterostyly in *O. pumila* from Taiwan. This incongruence may be caused by intraspecific polymorphisms in heterostyly, however, *O. pumila* from Taiwan investigated here also showed long-styled homostyly. In a bagging experiment, the reproductive success in fruit set and number of mature seeds was high, and comparable to open-pollinated individuals. In field observations, the rate of flower visitation was low, and no pollen grains were found on captured insects. Because of self-compatibility and high autodeposition efficiency independent of insect pollination, autogamy is thought to be the principal reproductive mechanism of *O. pumila* in the Ryukyus.

Key words: Autogamy, floral morphology, heterostyly, *Ophiorrhiza pumila,* Ryukyu Islands.

There are four taxa of *Ophiorrhiza* in the Ryukyus, *O. japonica* Blume var. *amamiana* Hatus., *O. japonica* Bl. var. *japonica*, *O. kuroiwai* Makino, and *O. pumila*. Among them *O. pumila* is the most frequently observed species in the area. *Ophiorrhiza pumila* Champ. ex Benth. (Rubiaceae) is a perennial herb, 5–20 cm in height, found in the Ryukyus, Taiwan, southern China, northern Vietnam, and the Philippines (Hatusima 1975, Liu and Yang 1998). In the Ryukyus, *O. pumila* is distributed in the south of Yakushima Isl., northern Ryukyus, and is very common in south of Amamioshima Isl., in the central Ryukyus. The plant bears white flowers approximately 5 mm long, and its cymose inflorescences consist of 1–10 flowers (Fig. 1). The genus *Ophiorrhiza* is heterostylous in general, as is typical for the Rubiaceae (Darwin 1976, Lo 1990, Liu and Yang 1998). In the Rubiaceae, 416 species in 91 genera are listed as heterostylous (Bahadur 1968), and there are more heterostylous species than in any other family of flowering plants (Ganders 1979, Faivre and McDade 2001). In Taiwan, *O. pumila* is reported as heterostylous (Liu and Yang 1998), but, Lo (1999) described *O. pumila* from continental China as having stigmata and anthers at almost the same height in corollas. Because of the disagreement between these reports from Taiwan and

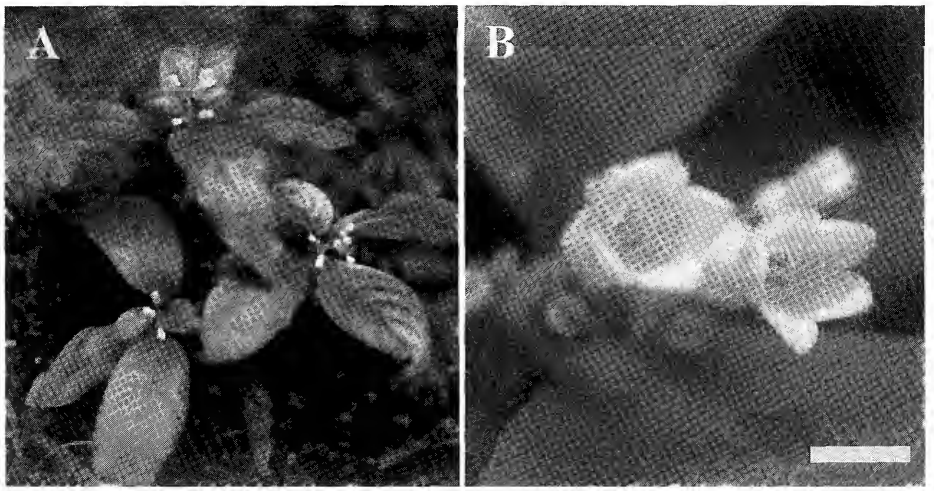

Fig. 1. Habit (A) and flowers (B) of *Ophiorrhiza pumila* in Mt. Nago, Nago-shi, Okinawajima Isl. on 4 June 2005. Scale bar = 3 mm.

continental China, it is of interest to determine whether *O. pumila* from the Ryukyus is heterostylous. There is, however, no detailed description of flower morphology of these plants. Here, we examined the floral morphology of *O. pumila* to clarify whether or not it is heterostylous in the Ryukyus.

Heterostyly is a reproductive mechanism that promotes animal-mediated legitimate pollinations, i. e., pollinations between stigmata of one floral morph and anthers at the equivalent level of another morph (Darwin 1877, Barrett and Glover 1985, Piper and Charlesworth 1986, Lloyd and Webb 1992), and the morphological traits are usually accompanied by a self-incompatibility system that prevents self- and intramorph fertilization (Barrett 1992). Thus, it is meaningful to examine the breeding system in practice to understand the heterostyly of a species. To do this, we performed field observations and conducted an experimental study of the breeding mechanism of *O. pumila*.

Materials and Methods

Floral morphology

Mature flowers of *Ophiorrhiza pumila* were collected during the flowering season from March to October of 2000 to 2005 in native habitats or in the greenhouse of the Faculty of Science, University of the Ryukyus, Okinawajima Isl., Japan. One to three mature flowers were randomly selected per plant. We also used specimens deposited in the herbarium of the Faculty of Science, University of the Ryukyus (RYU). In total, 109 individuals from 32 localities on five islands of the Ryukyus (Amamioshima, Tokunoshima, Okinawajima, Ishigakijima and Iriomotejima) and Taiwan were observed for style morph (Table 1). Collected flowers were preserved in 70 % ethanol or used directly for observation. Corolla tubes were cut and opened longitudinally with a needle, and the relative positions of stigma and anther heights in the corolla tubes were examined under a binocular microscope. A scanning electron microscope (JSM-6060LV, JEOL) was also used for detailed observation of some materials. Flowers were

Table 1. Collection localities of *Ophiorrhiza pumila*

Amamioshima Isl.	(Kagoshima Pref.): Shin-mura, Sumiyo-son, (1); Kawauchi Riv., Yamato-son, (1); Sumiyo Riv., Sumiyo-son, (1, 1[a]); locality unknown (2).
Tokunoshima Isl.	(Kagoshima Pref.): Mt. Inokawa, Tokunoshima-cho, (1); locality unknown (1[a]).
Okinawajima Isl.	(Okinawa Pref.): Benoki Riv. Kunigami-son, (1[b]); Mt. Terukubi, Kunigami-son, (1[b]); Ada, Kunigami-son, (1); Mt. Yonaha, Kunigami-son, (3, 1[b]); Mt. Ibu, Kunigami-son, (1[b]); Fun Riv., Kunigami-son, (2, 1[b]); Mt. Nekumachiji, Ogimi-son, (3); Ginoza, Ginoza-son, (1[b]); Mt. Tano, Nago-shi, (1[a]); Mt. Nago, Nago-shi, (1[a], 37[b]); Haneji, Nago-shi, (1); Mt. Kushi, Nago-shi, (2); Attabaru, Nago-shi, (3[a]); Sukuta, Nago-shi, (1[a]); Mt. Onna, Onna-son, (2); Mt. Ishikawa, Uruma-shi, (3[a]).
Ishigakijima Isl.	(Okinawa Pref.): Mt. Omoto, Ishigaki-shi, (4, 1[a]); Nagura Riv., Ishigaki-shi, (1[a]); Buzama, Ishigaki-shi, (1).
Iriomotejima Isl.	(Okinawa Pref.): Mt. Komi, Taketomi-cho, (2[b]); Urauchi Riv., Taketomi-cho, (1[a], 2[b]); Hinai-sara waterfall, Taketomi-cho, (1[a]); Hoshidate, Taketomi-cho, (1).
Taiwan	(mainland): Wulai Hsiang, Taipei Hsien, (1, 8[a]); Bard-wen, Taipei Hsien, (4); Mt. Yuanto, Ilan Hsien, (4).

Numerals in parentheses indicate the number of individuals examined in each locality. Individuals used for measurement of fruit set and number of mature seeds in bagging treatment ([a]) and in open-pollination ([b]) are also indicated. Specimens are deposited in the herbarium of the University of the Ryukyus (RYU).

dehydrated in an ethanol : t-butanol series and freeze-dried using a t-butanol freeze-drying device (VFD-21S, Vacuum Device, Inc.). Dried flowers were mounted on stubs and coated with gold using an ion sputterer (IB-3, Eiko), and observed using a SEM.

Fruit and seed production

To examine the breeding system of *Ophiorrhiza pumila*, especially concerning heterostyly, we conducted a bagging treatment to exclude pollinators and to assess selfing ability by autodeposition, i. e., autogamy. Twenty-three individuals with a total of 159 flower buds were bagged (Table 1). Fruiting was checked intermittently until the end of infructescence, and fruit-set was examined. Fruit set was defined as the proportion of flowers that developed into capsules containing mature seeds. Fruit-set for open-pollinated flowers was assessed by following the fate of 370 flowers of 47 marked individuals (Table 1). The number of mature seeds per fruit in the bagged and open treatments was also determined. Maturity of seeds was checked by germination test.

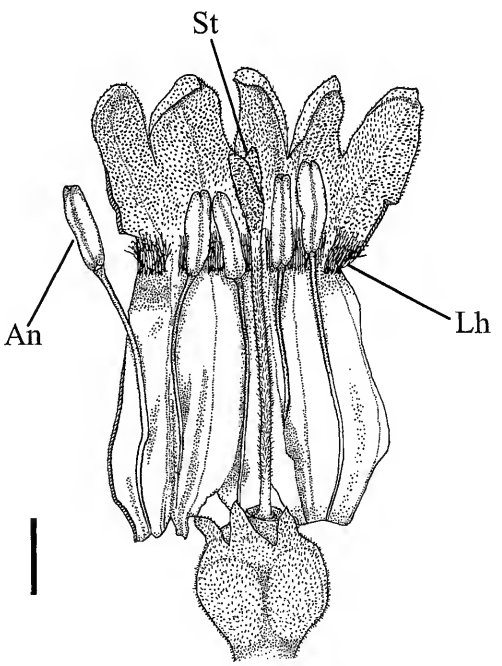

Fig. 2. A longitudinally opened flower of *Ophiorrhiza pumila* (Mt. Omoto, Ishigaki-shi, Ishigakijima Isl.; NK03308). Abbreviations: An, Anther; St, stigma; Lh, long-fascicled hairs. Scale bar = 1 mm.

Observation of floral visitors

In the Ryukyu Archipelago, *Ophiorrhiza pumila* is associated with subtropical broad-leaved evergreen forests dominated by *Castanopsis sieboldii* (Mak.) Hatus. *Ophiorrhiza pumila* occurs in mountainous moist habitats with moderate sunlight, for example, sides of hiking trails and banks of mountain streams. The present observations were conducted along a hiking trail on Mt. Nago (26°34′99″N, 128°00′43″E; elevation, 202 m), Nago-shi, Okinawajima Isl. We established a plot (2 × 5 m; sky openness, 10.9 %) along the hiking trail that included 132 individuals of *O. pumila*, including 29 flowering individuals. Direct observations of floral visitors were made during daylight for a total of 10 h on 3 days in June 2005, the middle of the flowering season. Insects that visited flowers were collected for identification and for assessment of the presence or absence of pollen grains of *O. pumila*. Collected insects were dried using silica gel, mounted on stubs, coated with gold, and examined using SEM.

Results

Floral morphology

In all individuals of *Ophiorrhiza pumila* examined from the Ryukyus and Taiwan, stigmata and anthers were found at almost the same height around the throat of corolla tubes (Fig. 2). Stigmata were included in, or scarcely exsert from corollas. Stigmata of *O. pumila* were bilobed, and stigmatic lobes were ovate to narrowly ovate, although they were sometimes coherent with each other and appeared claviform. The tips of the stigmatic lobes exceeded anthers slightly; however, the base of the stigmatic lobes did not exceed the anthers. At the throat of corolla tubes, long-fascicled hairs were observed at the same height as the anthers (Fig. 2). These hairs, however, were not so long as to cover

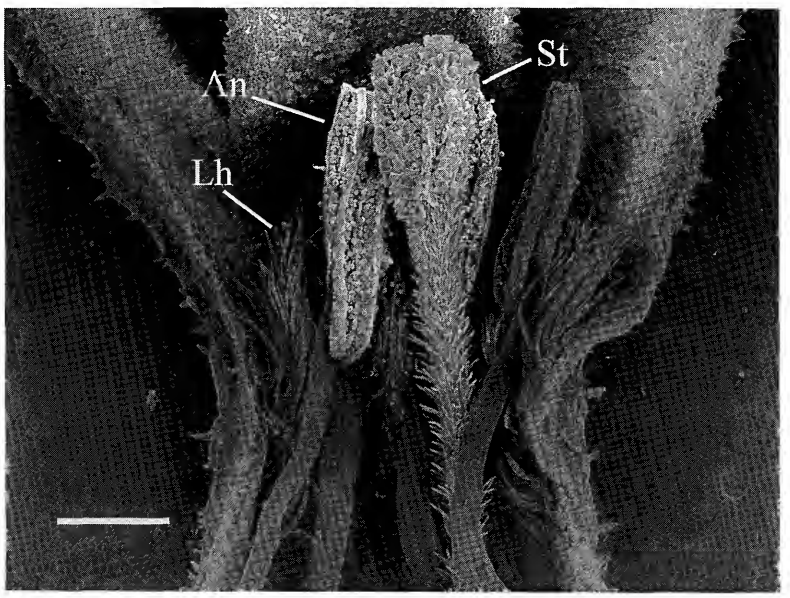

Fig. 3. Scanning electron micrograph of a longitudinally opened flower of *Ophiorrhiza pumila* (Mt. Yuanto, Ilan Hsien, Taiwan; NK05778). Abbreviations: An, Anther; St, stigma; Lh, long-fascicled hairs. Scale bar = 500 μm.

Table 2. Fruit set and number of mature seeds per fruit (mean ± SD) in bagging treatment and in open-pollination

Treatment	N*	Flowers	Fruits	Fruit set (%)	Mature seeds
Bagging	23	159	152	95.9 ± 10.3	89.0 ± 22.8
Open-pollination	47	370	341	94.4 ± 8.3	91.6 ± 35.2

*Number of individuals.

Table 3. Flower-visiting insects and presence/absence of pollen grains of *Ophiorrhiza pumila* on them

Species	presence/absence of pollen grains
Hymenoptera, Halictidae	
Lasioglossum sp.	absent
Diptera, Syrphidae	
Baccha nubilipennis	absent
Epistrophe balteata	absent

the whole length of the anthers. Thus, stigmata and anthers came in touch with each other (Fig. 3). Anthers sometimes adhered to stigmata and deposited pollen grains on them.

Fruit and seed production

In the bagging treatment, 152 of 159 flowers developed into capsules with mature seeds, while in the open, 341 of 370 flowers did so (Table 2). Thus, fruit sets (mean ± SD) were 95.9 ± 10.3 % and 94.4 ± 8.3 % in the bagged and open treatments, respectively. There was no significant difference in fruit set between treatments (Mann-Whitney U test, $P > 0.05$). The number of mature seeds per fruit was 89.0 ± 22.8 and 91.6 ± 35.2 in the bagged and open treatments, respectively (Table 2). Again, there was no significant difference in number of mature seeds between treatments (Mann-Whitney U test, $P > 0.05$).

Flower-visiting insects

Three species of insects (a small bee and two syrphid flies) visited the flowers of *Ophiorrhiza pumila* during the 10 h of observation (Table 3): *Lasioglossum* sp. (Halictidae), *Baccha nubilipennis* (Syrphidae), and *Epistrophe balteata* (Syrphidae). All the visitations were observed during 10 am–12 pm. However, no pollen grains of *O. pumila* were observed on the three insect species (Table 3).

Discussion

The flowers of *Ophiorrhiza pumila* are not distylous, but rather monomorphic, with stigmata and anthers at almost the same height around the throat of corolla tubes. Although the tip of the stigmatic lobes exceeds anthers slightly, most parts of the stigmata and anthers are at equal height, and therefore, the floral morph of *O. pumila* is not long-styled. This is clear in comparison to the long-styled morph of congeneric *O. japonica* var. *japonica*, whose stigmata obviously exceed the anthers (Nakamura et al. unpublished data). Thus, the floral morph of *O. pumila* is defined as long-styled homostyly. This agrees with Lo's (1999) description of similar heights of stigmata and anthers in *O.*

pumila, but differs from the description of Liu and Yang (1998), who reported *O. pumila* as heterostylous. Geographical intraspecific polymorphism in heterostyly have been reported in some taxa, e. g., *Eichhornia paniculata* (Pontederiaceae) in Brazil, Jamaica, and Nicaragua (Barrett et al. 1989, Barrett and Husband 1990), and *Turnera ulmifolia* (Turneraceae) in the Caribbean (Barrett and Shore 1987). Disagreement between Lo (1999), this study, and Liu and Yang (1998) regarding the presence or absence of heterostyly in *O. pumila* may be caused by geographical polymorphism if *O. pumila* is monomorphic and homostylous in continental China and the Ryukyus, while heterostylous in Taiwan. However, we investigated 17 specimens from three localities in Taiwan, and all indicated long-styled homostyly. An intensive survey of numerous specimens from additional localities in Taiwan is necessary to clarify the presence or absence of heterostyly in Taiwan and, if it exists, the geographical trend of the floral morphology.

Correlation between the breakdown of heterostyly and intraspecific polyploidy has been reported in some plants (Barrett and Shore 1987, Barrett 1988, Naiki and Nagamasu 2004). However, the chromosome number of *O. pumila* from the Ryukyus was examined and reported to be diploid, with $2n = 22$ (Nakamura et al. 2003). Long-styled homostyles appear more frequently than do short-styled homostyles (e. g., *Primula*, Ernst 1955; *Amsinckia*, Ray and Chisaki 1957). Dowrick (1956) suggested that long-styled homostyles spread more rapidly than do short-styled homostyles when each morph is introduced singly into a heterostyled population under certain conditions, e. g., low rate of pollinator visitation. Long-styled homostyly of *O. pumila* from the Ryukyus may be an example of this. On Mt. Nago, only three insect individuals visited *O. pumila* in 10 h of observation, and they carried no pollen grains after visitation. The Ryukyus lie at the northern limit of the distribution range of *O. pumila*, which could lead to a lack of effective pollinators that may be found in more southerly parts of its distribution. A detailed quantitative study of the pollination ecology of *O. pumila* over its whole range of distribution may reveal the cause of long-styled homostyly in this species.

During examinations of floral morphology, we found that some flowers had pollen grains from their own anthers deposited on the stigmata. This phenomenon was observed not only in bagged individuals, but also in individuals in native habitat for flowers collected in the field. Self-pollination in distylous taxa can result in stigma clogging, which reduces reproductive success (Darwin 1877, Lloyd and Webb 1992). However, bagged flowers of *O. pumila* showed high fruit-set and produced many seeds, equivalent to that of open-pollinated individuals. High reproductive success in the bagged treatment indicates self-compatibility and high autodeposition efficiency in this species, independent of insect pollination. During the flowering season of *O. pumila* at the study site, some other plants flowered concurrently: *Alpinia intermedia* Gagnep. (Zingiberaceae), *Antidesma pentandrum* (Blanco) Merr. (Euphorbiaceae), *Ardisia pisilla* DC. (Myrsinaceae), *Psychotria rubra* (Lour.) Poir. (Rubiaceae), and *Scutellaria rubropunctata* Hayata (Labiatae). In particular, *Alpinia intermedia* bore many outstanding white flowers with strong scent, and therefore, many flower-visiting insects concentrated on those flowers. Inflorescences of *O. pumila* are less conspicuous because of the small, unscented flowers and the low height of the plant. Considering the low rate of flower visitation and the absence of pollen grains on the visiting insects in the natural condition in contrast to self-compatibility and high autodeposition efficiency, autogamy is thought to be the principal reproduc-

tive mechanism of *O. pumila*.

We express our sincere gratitude to Dr. G. Kokubugata (Tsukuba Botanical Garden, The National Science Museum, Tokyo), Mr. T. Kudaka (Takamori Orchid Museum), and Dr. S. Matsumura (University of the Ryukyus) for their kindness in providing plant materials and valuable suggestions. This study was supported in part by a Grant-in-Aid for Young Scientists (B) (no. 15770061) for T. D., and by a Research Fellowship for Young Scientists from the Japan Society for the Promotion of Science to K. N. This study was also partially supported by a grant for the 21st Century COE program of the University of the Ryukyus "The Comprehensive Analyses on Biodiversity in Coral Reef and Island Ecosystems in Asia and Pacific Regions" from the Ministry of Education, Culture, Sports, Science, and Technology, Japan (Monbukagakusho).

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中村 剛[a]，傳田哲郎[b]，横田昌嗣[b]：**琉球列島におけるチャボイナモリ（アカネ科）の等花柱性と自動的自家受精**

琉球列島産チャボイナモリについて，異型花柱性の有無に関して花形態を調査した．その結果，チャボイナモリは，柱頭と葯が花筒喉部付近でほぼ同じ高さにある等花柱性で，二型花柱性を持たないことがわかった．この結果は，中国大陸産チャボイナモリについてのこれまでの記述と一致したが，台湾産チャボイナモリが異型花柱性であるとする記述とは異なった．この不一致は，チャボイナモリの異型花柱性に地理的な種内変異があるためかも知れない．しかし，本研究で調査した台湾産チャボイナモリはいずれも等花柱性であった．袋がけ実験の結果，チャボイナモリの結実率，成熟種子数は，袋がけをしていない野外個体と同等に高い値を示した．一方，野外観察において，チャボイナモリへの昆虫の訪花はまれで，その体表にチャボイナモリの花粉は認められなかった．自家和合性と虫媒によらない高い自家受粉率を考えると，チャボイナモリの主要な繁殖様式は自動的自家受精であると考えられる．

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